FCS15 - SOP for Quantitation of Heroin using GC-FID

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1. Scope

1.1. This document establishes the procedures for quantifying heroin in samples containing heroin for use in case work and reporting out quantifiable results of heroin in test samples using Gas Chromatography Flame Ionization Detector (GC-FID) instrumentation.

2. Background

2.1. This method is based off the document provided by the Drug Enforcement Agency (DEA) method for *Quantitation of Heroin* (2016) using GC-FID. This document provides guidance and is in support of the *Forensic Science Laboratory (FSL) Quality Assurance Manual (QAM)* and conforms to the requirements of the accreditation standards under ISO/IEC 17025 (current revision) and the supplemental standards.

3. Safety

3.1. Reagent Toxicity:

3.1.1. Personnel should refer to the appropriate SDS for solvents and reagents used during analysis for any specific safety requirements.

3.1.2. For a complete review of required Health and Safety regulations of the FSL, see *DOM13 -DFS Health and Safety Manual.*

3.2. Protective Equipment:

3.2.1. Personnel should wear personal protective equipment (PPE) including

lab coat, gloves, and safety goggles when carrying out standard operating procedures.

3.2.2. Wear vinyl or nitrile gloves when handling these chemicals to prevent absorption through the skin. If any chemicals are spilled onto gloves, discard gloves into hazardous waste.

3.3. Training:

3.3.1. Formal training in use of instruments and software is necessary.

3.4. Personal Hygiene:

3.4.1. Universal Precautions shall be followed. Care should be taken when handling chemicals or any biological specimen. Routine use of gloves and proper hand washing should be practiced.

3.4.2. Refer to *DOM13 – DFS Health and Safety Manual.*

3.5. Disposal of Waste:

3.5.1. Waste materials shall be disposed of in compliance with laboratory, Federal, state, and local regulations. Solvents and reagents should always be disposed of in an appropriate container clearly marked for waste products and temporarily stored in a chemical fume hood.

3.5.2. Consult DFS Safety Officer for proper procedures.

4. Materials / Equipment Required

4.1. Reagent Grade Acetonitrile (ACN), Methanol (MeOH), and Chloroform ($CHCl_3$), or higher purity.

4.2. Ultra-High Purity Helium tanks

4.3. Hydrogen generator or tanks for the FID

4.4. Gas Chromatography Flame Ionization Detector (GC-FID), fully assembled, including: solvent vials, injection syringe, and other consumables.

4.5. Glassware

4.5.1. **Note:** Glassware used for quantitative measurements shall be Class A and calibrated with traceability to the International System of Units (SI)):

4.5.1.1. Volumetric flasks

4.5.1.2. 1-10 mL volumetric pipettes

4.5.1.3. Other volumetric glassware, as appropriate

4.6. Balances

4.6.1. **Note:** Balances used for quantitative measurements shall be calibrated with traceability to SI and have a five decimal place minimum resolution.

4.7. Binder/Folder for standard results, or electronic equivalent

4.8. Maintenance Logbooks and Control Charts, or electronic equivalent

5. Standards and Controls

5.1. Analyte standards shall be purchased from an approved vendor with established metrological traceability (i.e., from an ISO 17034 accredited provider).

5.1.1. Heroin hydrochloride (HCl) (solid powder)

5.2. Tetracosane (solid powder) (internal standard)

5.3. Mannitol (solid powder) (for dilution)

5.4. Internal Standard Solution (ISTD), 0.4000 mg/mL tetracosane (exact value needed):

5.4.1. About 100mg tetracosane in a 250 mL volumetric flask, dilute to mark with chloroform/methanol (9:1). Other volumes are acceptable as long as ratio is maintained.

5.5. Annual Calibration Curve Evaluation solutions:

5.5.1. Heroin HCl Calibration Stock Solution, about 5 mg/mL of 100% Heroin (exact value needed using purity):

5.5.1.1. About 250 mg Heroin HCl in tared, 50 mL volumetric flask. Other volumes are acceptable as long as ratio is maintained.

5.5.1.2. Dilute to mark with ISTD solution.

5.5.2. Heroin HCl Calibration Dilutions: (dilute to mark with ISTD solution; exact value needed using purity)

5.5.2.1. A nine-point calibration curve is used to assess linearity and to establish a working range for the method. The following concentrations are suggested for each calibration point, however, other concentrations are acceptable as long as the working range is covered.

5.5.2.1.1. Cal 1: (1:50) about 0.1 mg/mL

5.5.2.1.2. Cal 2: (1:10) about 0.5 mg/mL

5.5.2.1.3. Cal 3: (2:10) about 1.0 mg/mL

5.5.2.1.4. Cal 4: (3:10) about 1.5 mg/mL

5.5.2.1.5. Cal 5: (4:10) about 2.0 mg/mL

5.5.2.1.6. Cal 6: (5:10) about 2.5 mg/mL

5.5.2.1.7. Cal 7: (6:10) about 3.0 mg/mL

5.5.2.1.8. Cal 8: (8:10) about 4.0 mg/mL

5.5.2.1.9. Cal 9: Heroin HCl Stock Solution (about 5.0 mg/mL)

5.6. Calibrant Solution (for two-point recalibrations):

5.6.1. Heroin HCl (>99% purity) diluted with ISTD solution at 1.0 mg/mL (for example, 25.00 mg of Heroin weighed in a tared 25 mL volumetric flask)

5.6.2. A lot number shall be assigned in the following format:

5.6.2.1. Initialism of Quality Control Heroin (QCH)

5.6.2.2. Designation of Calibrant (CAL)

5.6.2.3. Date in YYYYMMDD format

5.6.2.4. Initials of preparer

5.6.2.5. Example: QCHCAL20221025LN

5.6.3. The assigned expiration shall be the expiry date of the Heroin standard used.

5.6.4. All new calibrant preparations shall be checked for accuracy and documented prior to being used in casework (i.e., run in a sequence and checked against QC solutions to meet criteria outlined in Table 2).

5.6.5. All new calibrant preparations shall be documented in a Quant Reagent Logbook. Documentation shall include lot numbers, weights and volumes, concentration, date of preparation, identity of the preparer, and the expiration date.

5.7. Quality Control Standards

5.7.1. Two Quality Control Standard solutions are made and evaluated over time to assess performance of the method. These solutions are made from a heroin mixture at concentrations of the low end and high end of the working range.

5.7.2. The bulk QC Heroin mixture is made:

5.7.2.1. Heroin HCl (>99% purity) added to sucrose or mannitol to reach approximately 65% Heroin purity (or approximate average representative purity of street samples)

5.7.2.2. Mix contents and grind in mortar

5.7.2.3. A lot number shall be assigned in the following format:

5.7.2.3.1. Initialism of Heroin Mixture (HM)

5.7.2.3.2. Date in YYYYMMDD format

5.7.2.3.3. Initials of preparer

5.7.2.3.4. Example: HM20221025LN

5.7.3. Quality Control (QC) standard solutions (for example):

5.7.3.1. QC Low – About 12 mg bulk QC Heroin Mixture, added to 25 mL volumetric flask, dilute to mark with ISTD solution.

Resulting concentration: 0.3 mg/mL

5.7.3.2. QC High – About 135 mg bulk QC Heroin Mixture, added to 25 mL volumetric flask, dilute to mark with ISTD solution. Resulting concentration: 3.5 mg/mL

5.7.3.3. Filter prior to injection.

5.7.3.4. A lot number shall be assigned in the following format:

5.7.3.4.1. Initialism of Quality Control Heroin (QCH)

5.7.3.4.2. Designation of Low or High

5.7.3.4.3. Date in YYYYMMDD format

5.7.3.4.4. Initials of preparer

5.7.3.4.5. Example: QCHLOW20221025LN

5.7.3.5. The assigned expiration date of each QC solution shall be three months from the preparation date, or the expiry date of the heroin standard used, whichever comes first.

5.7.3.6. All new preparations of QC solutions shall be checked for accuracy and documented (i.e., run in a sequence and meeting acceptance criteria outlined in Table 2) prior to being used in casework.

5.7.3.7. All bulk QC Heroin Mixture and QC standard solution preparations shall be documented in a Quant Reagent Logbook. Documentation shall include lot numbers, weights and volumes, concentration, date of preparation, identity of the preparer, and the expiration date.

6. Calibration

6.1. Annual Evaluation of Calibration Curve

6.1.1. An average of five responses are taken at each calibration level

6.1.2. The average response of each concentration level is compared to the actual concentration, with an average sensitivity calculated at each level (see section 9 for calculations).

6.1.3. For the concentration curve to be acceptable, the average sensitivity of each calibration level shall be within 5% of the overall average sensitivity.

6.1.4. While in use for casework, approved calibration Excel worksheets will be saved electronically.

6.1.5. Once a curve is no longer suitable for casework, the respective Excel worksheet will be archived in the appropriate electronic folder.

6.1.6. This calibration curve will be run at least once per year or as needed

(based on column, environment, or method changes) to evaluate the suitability of the column for quantitation and a re-establishment of working range.

6.2. Weekly Calibrant Run with Samples

6.2.1. A calibrant of heroin standard at approximately 1 mg/mL shall be run, at minimum, once per week that heroin purity analysis is performed. For optimal results, a calibrant should be run with each batch sequence.

6.2.1.1. A calibrant shall also be run for any new weekly sequence created after performance of major maintenance that may modify the function of the equipment (i.e., changing of major parts, column trimming, etc.). (See *FCS09 – SOP for Operating and Maintaining GC-MS and GC-FID Instruments*)

6.2.2. Two quality control standards (QC Low and QC High) shall be run for each batch to assess the accuracy of the calibration curve.

6.2.3. The calibrant will be used to establish the calibration curve for that sequence's analysis (until another calibrant is run).

6.2.3.1. The origin and the response from the calibrant will be the two points used to determine the new calibration curve between calibrant runs.

6.2.4. The calibrant response will be monitored in an electronic control chart.

7. Procedures

7.1. Heroin quantitation is run using the FCU_HQUANT method, or another validated method, with Gas Chromatograph Flame Ionization Detection (GC-FID)

7.2. Acceptable Method Parameter Variations (make mention in case notes):

7.2.1. Increase flow and temperature ramp at 2.5 minutes (past the retention times of both the analyte and internal standard) to remove late eluting compounds (*e.g.*, diltiazem, noscapine, …), as per analyst's discretion.

7.2.2. Sample preparation solvent of chloroform or chloroform/methanol (9:1), as per analyst's discretion.

7.2.3. Each linearity concentration may be injected in lowest-to-highest or highest-to-lowest order, as per analyst's discretion.

7.2.4. Accuracy and recovery may be calculated from either a 3-point or 9-point curve, as per analyst's discretion.

7.3. Unknown Sample Preparation

7.3.1. Prepare two separate samples (three if necessary) for casework.

7.3.1.1. Accurately weigh the sample and record weight values using a

five-place balance (reading down to 0.00001g). Document sample weight in case notes.

7.3.1.2. Dissolve in the ISTD solution using appropriate volumetric glassware.

7.3.1.3. Add sufficient quantity to result in a concentration that is within the working range of this method. Example: weigh approximately 10mg of sample and dilute with 2mL of ISTD.

7.3.1.4. Filter prior to injection.

7.3.2. Run samples on GC-FID

7.3.2.1. Each batch sequence shall include a negative control, calibrant (if necessary), and two QCs (low and high). The batch sequence should generally be run as follows:

7.3.2.1.1. Blank

7.3.2.1.2. Calibrant

7.3.2.1.3. QC Low

7.3.2.1.4. Sample 1

7.3.2.1.5. Sample 2

7.3.2.1.6. Additional samples (if applicable) (Note: Multiple case samples may be run within the same batch)

7.3.2.1.7. QC High

7.3.2.1.8. Blank

7.3.3. Generated data shall be entered into the FCU Heroin Purity Worksheet (Document Control Number 30400) to calculate the percentage of Heroin. Calculations performed using this worksheet are outlined in Section 9.

7.3.3.1. Note: Calculations generated by the worksheet shall be manually checked for accuracy using formulas in Section 9.

7.4. **Acceptance Parameters** – The following are the acceptance parameters for the GC-FID.

Table 1: GC-FID Acceptance Criteria

	Acceptance Criteria	Detail
GC-FID PARAMETERS	**Retention Time**	Retention time of analyte peak must match within 2% of standard.
	S/N* Cut Off	Analyte peak must be

		more than three (3) times greater than noise.
	Peak width resolution	Analyte peak must be baseline resolved, as evaluated by the analyst.

*S/N = Signal-to-Noise Ratio

Table 2: Calibration Curve and Sample Acceptance Criteria

	Acceptance Criteria	**Detail**
CALIBRATION CURVE AND SAMPLE ACCEPTANCE	**Check standard falls within tolerance**	Tolerance set to 5%
	Calibration curve is still valid	Calibration curve (two-point recalibration) is applicable for all casework run within the same week
	Two samples of the same item are within 10%	Absolute difference in quant of two samples of an item must be under 10%
	Range of >10% between samples requires third quantitation	Analyst shall average the sample concentrations

7.4.1. QC Check Acceptance

7.4.1.1. Positive control checks shall be within 5% of the expected value be acceptable.

7.4.1.2. If a QC check value does not fall within this range, a new standard solution may be prepared and injected.

7.4.1.3. If more than one QC check value does not fall within this range, a new calibration curve shall be prepared and used.

7.5. Control Chart and Logbook Maintenance

7.5.1. All calibrant and QC responses for each sequence shall be entered into an electronic control chart. Significant parameters include peak retention time, response signals, calibration slope, and QC purity values.

7.5.2. All calibrant and QC runs along with corresponding blanks for each sequence shall be filed or scanned and maintained in an electronic logbook.

7.6. Reporting of Results

7.6.1. Heroin purity results shall be reported with the associated uncertainty value and corresponding confidence level.

7.6.2. After quantitation, the heroin result will not be reported if the results fit any of the following criteria:

7.6.2.1. The purity determination is below the detectable limit.

7.6.2.2. The purity determination is below the reported uncertainty value.

7.6.3. When heroin is detected but not quantitated, a note will be made on the report stating the reason why quantitation was not performed or why a quantitation result was not reported.

7.6.4. Deviations are acceptable but shall be recorded in case notes and follow agency policy of deviations.

8. Sampling

8.1. Perform sampling plan as outlined in *FCS01 – SOP for Detecting Controlled Dangerous Substances* and *FCS02 – SOP for General Laboratory Procedures for FCU*. Note: Heroin quantitation will not be performed on samples that fit any of the following criteria:

8.1.1. The total net weight of the sample is less than 100 mg before analysis (or, in the case of multiple homogenous units, estimated net weight of all possible units).

8.1.2. The heroin is a very minor component in the sample, as evaluated by the analyst (based on the relative abundance in GCMS. The estimated cutoff is less than 5% of the largest peak).

8.2. A composite of multiple samples may be necessary to meet the minimum weight required to perform quantitative analysis (100 mg). In cases where a composite shall be made, the chemist will first test each of the selected samples with a screening technique prior to making a composite (Category A, B, C). The number of items composited shall be documented in the case notes. An appropriate number of units will be composited until the 100 mg minimum is met.

9. Calculations

9.1. Linearity Evaluation of Calibration Standards

9.1.1. The following is an example calculation and evaluation of response values for the 9 calibrators used in this method, using Excel.

LINEARITY EVALUATION

Method Name: FCS15 - SOP for Quantitation of Heroin using GC-FID
Target Compound: Heroin
Laboratory: DC Department of Forensic Sciences, 401 E St SW, Washington, D.C. 20024
Instrument ID: D3

Concentration (mg/mL)	Cal Dilution Number	Analyte Response 1	Analyte Response 2	Analyte Response 3	Analyte Response 4	Analyte Response 5	ISTD Response 1	ISTD Response 2	ISTD Response 3	ISTD Response 4	ISTD Response 5
0.1004	1	6.04510	6.07659	6.04413	6.06957	6.07616	44.41050	44.66372	44.41793	44.68648	44.78850
0.5021	2	31.04008	31.38504	31.06693	31.15440	31.46083	44.61116	45.11726	44.69828	44.72971	45.28262
1.0042	3	62.35224	61.91711	61.60768	61.62232	62.17142	45.38428	45.18236	44.81511	44.92493	45.31451
1.5064	4	91.11919	91.44414	92.23016	91.76097	92.83170	44.32288	44.55284	44.94694	44.50371	45.35350
2.0085	5	124.38412	124.95324	125.13978	124.00094	124.26001	44.81717	45.09495	44.94883	44.68200	44.84151
2.5106	6	153.53494	153.42752	152.57861	153.66118	152.04192	44.91734	44.81447	44.70959	44.86489	44.58757
3.0127	7	179.27669	183.62903	181.91058	185.49750	184.89232	43.83622	44.95296	44.78127	45.38866	45.25607
4.0170	8	242.70372	244.92767	246.61592	245.56288	245.43718	44.86417	44.91528	45.41814	45.09751	44.86969
5.0212	9	297.44800	303.58475	301.94946	303.58536	303.87320	43.29699	44.02341	43.86954	43.89145	43.99567

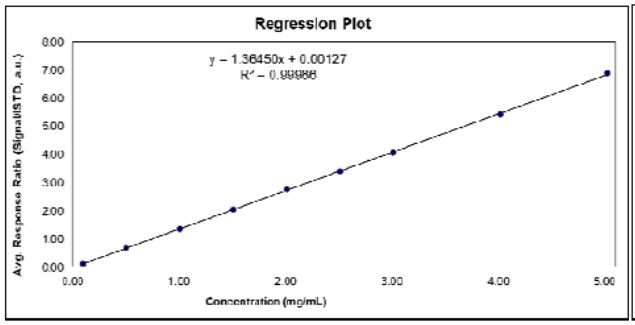
Concentration (mg/mL)	Cal Dilution Number	Average Response Ratio (Analyte/ISTD)	Sensitivity	Overall Avg. Sensitivity	95% Limit	105% Limit	Result
0.1004	1	0.13595	1.35405	1.36614	1.29783	1.43445	Pass
0.5021	2	0.69555	1.38527	1.36614	1.29783	1.43445	Pass
1.0042	3	1.37253	1.36679	1.36614	1.29783	1.43445	Pass
1.5064	4	2.05380	1.36338	1.36614	1.29783	1.43445	Pass
2.0085	5	2.77532	1.38179	1.36614	1.29783	1.43445	Pass
2.5106	6	3.41788	1.36138	1.36614	1.29783	1.43445	Pass
3.0127	7	4.08183	1.35487	1.36614	1.29783	1.43445	Pass
4.0170	8	5.44158	1.35464	1.36614	1.29783	1.43445	Pass
5.0212	9	6.89449	1.37308	1.36614	1.29783	1.43445	Pass

SUMMARY

Concentration (mg/mL)	Average Response Ratio (Analyte/ISTD)	Sensitivity
0.10040	0.13595	1.35405
0.50210	0.69555	1.38527
1.00420	1.37253	1.36679
1.50640	2.05380	1.36338
2.00850	2.77532	1.38179
2.51060	3.41788	1.36138
3.01270	4.08183	1.35487
4.01700	5.44158	1.35464
5.02120	6.89449	1.37308

Corr. Coefficient:	0.999857617
y-intercept:	0.00126671
Slope:	1.364495986

LINEAR RANGE: 0.1004 mg/mL to 5.0212 mg/mL



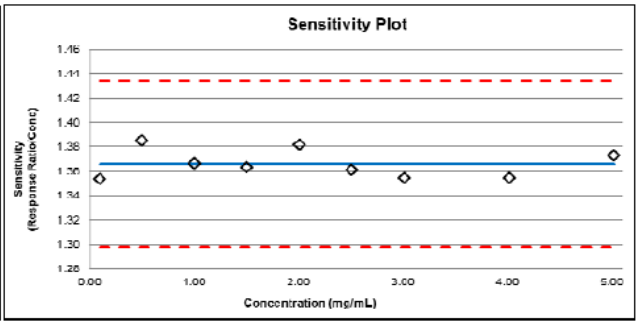


Figure 1. Example calculation and evaluation of calibration standards for this method.

9.1.2. Response Ratio = Analyte Response / ISTD Response

9.1.3. Sensitivity = Average Response Ratio / Concentration

9.2. Calculation of Heroin Purity

9.2.1. From the generated data, divide the heroin peak corrected area by the internal standard's peak area to calculate the area ratio.

9.2.2. Using the two-point recalibrated calibration curve (from section 6.2), solve for the heroin concentration.

9.2.3. To calculate the percent heroin:

9.2.3.1. Slope of Calibration Curve = (Area of heroin calibrant peak) / (Area of internal standard calibrant peak) (a.u.) x (Concentration of internal standard) / Concentration of heroin Calibrant (mg/mL))

9.2.3.2. Percent Purity = 100% x (Area of sample heroin peak (a.u.) x Concentration of Internal Standard (mg/mL)) / (Slope of Calibration Curve x Area of sample internal standard peak (a.u.) x Total Concentration of Sample (mg/mL)

9.2.4. Regarding the quantitation, or purity, analysis of test items for heroin, the standard result will be assuming a salt form of “heroin hydrochloride” or “heroin HCl.” The calibration curve on the gas chromatogram flame ionization detector (GC-FID) instrument will generate percentages assuming the heroin HCl. In instances where a purity is requested assuming a heroin base, or vice versa, a conversion factor may be used. This value is the ratio of the molecular weight of the heroin base to heroin hydrochloride (hydrated), or:

9.2.4.1. Calculate Heroin Base from Heroin Hydrochloride

9.2.4.1.1. Purity (heroin base) = Purity (heroin HCl) x (369.417g/mol heroin) / (423.8902 heroin HCl [H2O])

9.2.4.1.2. Purity (heroin base) = Purity (heroin HCl) x 0.871492

9.2.4.2. Calculate Heroin Hydrochloride from Heroin Base

9.2.4.2.1. Purity (heroin HCl) = Purity (heroin base) x (423.8902 heroin HCl [H2O]) / (369.417g/mol heroin)

9.2.4.2.2. Purity (heroin HCl) = Purity (heroin base) x 1.147457

10. Uncertainty of Measurement

10.1. Uncertainty of measurement is calculated based on the following factors:

10.1.1. Process Reproducibility

10.1.1.1. Process uncertainty accounts for user and environmental variations, based on quality control data.

10.1.2. Balance Uncertainty

10.1.2.1. Balance uncertainty accounts for the uncertainty of the balances used for measuring weights of calibrants and samples.

10.1.3. Volumetric Uncertainty

10.1.3.1. Volumetric uncertainty accounts for the uncertainty of glassware used for measuring calibrant and sample volumes.

10.1.4. Standard Purity Uncertainty

10.1.4.1. Standard purity uncertainty accounts for purity uncertainty associated with standards used for calibrant preparations.

10.2. Uncertainty of measurement is assessed on an annual basis.

10.3. See *FCS21 – Procedure for Uncertainty in Measurement*, for purity and weight uncertainty calculations.

11. Limitations

11.1. Not applicable

12. Documentation

12.1. Quant Reagent Logbook

12.2. Heroin Purity Worksheet (Document Control Number 30400)

12.3. Instrument Logbooks and Control Charts

13. References

13.1. *Quantitation of Heroin*, Drug Enforcement Agency, Nov. 28th, 2016.

13.2. *Quantifying Heroin by GC-FID with Internal Standard*, Austin Police Department, Forensic Chemistry Section, Technical Manual, Jan. 1, 2014.

13.3. FCS01 – SOP for Detecting Controlled Dangerous Substances

13.4. FCS02 – SOP for General Laboratory Procedures for FCU.

13.5. FCS09 – SOP for Operating and Maintaining GC-MS and GC-FID Instruments

13.6. FCS21 – Procedure for Uncertainty in Measurement

13.7. Forensic Science Laboratory Quality Assurance Manual (current revision).

13.8. ISO/IEC 17025 – General Requirements for the Competence of Testing and Calibration Laboratories, International Organization for Standardization, Geneva, Switzerland (current revision)

13.9. American National Standards Institute National Accreditation Board Supplemental Requirements (current revision)